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Study the effect of compound Extracted from pomegranate peels and green tea on some biochemical variables in rabbit serum induced with Myocardial Infarction

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ABSTRACT

This study aims to investigate the effect of compounds extracted from pomegranate peels and green tea on some biochemical variables in the serum of domestic male rabbits with recently myocardial infarction.

The study was conducted in the Animal House of the College of Veterinary Medicine. 24 male rabbits were used, with an average of weights ranging between 1800 - 2000 grams, which were divided into three groups (8 / group). The first group was fed a standard diet as a control group and the second and third group fed a standard diet added to it Cholesterol by 1%. After 45 days, the second and third group animals were dosed, in which the myocardial infarction developed the extracted compounds, and the myeloperoxidase enzyme activity and the level of troponin, potassium, and magnesium were measured.

Blood samples were collected from the control groups, the second and the third in which the disease was developed, and after 21 days the samples were collected from the second and third groups after treatment with the extracted compounds. The induction of myocardial infarction led to a significant increase in the activity of myeloperoxidase enzyme MPO and the level of troponin, potassium and magnesium, with a probability level of $p \leq 0.01$.

In comparison with control, there was a significant decrease in myeloperoxidase activity and potassium troponin level for groups in which the extracted compounds were dosed after disease onset, and with a probability $p \leq 0.01$ compared to the affected groups.

Introduction

Cardiovascular disease (CVD) is a disease that affects millions of people of all ages around the world, as it is the main cause of death, and current expectations indicate that for every three injuries there is one death, and early and accurate diagnosis gives information about the extent of the seriousness Injury, and the chance of survival of the victim, which reduces the damage to the development of the condition through which the heart muscle is exposed to complications in the future, in addition to CVD, there are several types of heart injuries, including heart failure Chronic and acute coronary syndrome such as Myocardial Infraction (MI) [1-4], Cardiovascular disease symptoms may not appear until sometime after the injury or in advanced stages of the disease [5].

Any defect in the heart muscle or the ability of the heart muscle to contract, which causes a temporary or permanent decline in the ability of the heart to contract, there will be a decrease in the pumping of blood to the vital organs in the body[6]. Through this, pathophysiology can be identified in order to reach a diagnosis of the disease, monitor it and prevent its development. It is noticeable that coronary artery disease leads to myocardial infarction and a heart attack that leads to sudden death, and this happens to an imbalance between oxygen demand and its supply, the increased demand may cause an increase in heart rate due to other pathophysiological phenomena such as left ventricular contraction and heart wall tightening and contraction. The reduced oxygen supply most often occurs due to coronary artery

spasms and coronary artery disease. Therefore, myocardial infarction clinically appears as Angina pectoris [7].

Medicinal plants and herbal remedies occupy an important position in the sciences of medicine and pharmacy, because it is considered a safe source for the pharmaceutical industry. The medicinal plant is a plant that contains in one or more of its different parts one or more effective chemicals in low or high concentration and has the ability to treat a specific disease or reduce Symptoms of infection for this disease if it is given to the patient in its pure extracted form or in the form of fresh or dried natural vegetable herb. The scientist (Dragendroff) knew in his definition of the medicinal plant as (that everything of plant origin is used medicinally, it is a medicinal plant), and possesses medicinal plants used as antibiotics and bacteriostatic, or as painkillers, there are many effective compounds such as: alkaloids, tannins, soaps, volatile oils, flavonoids, saliva, gums and phenolic compounds [8].

Materials and methods

Extraction and separation of the A1 plant extract from the pomegranate peel: the peel extract of the pomegranate fruit has been prepared using a continuous extraction device (soxhlet) depending on the nature of the active compounds separated from the plant and the nature of the solvent used in the separation process and by using the successive solvent system, taking into account the boiling point of the solvent used, have been collected. Pomegranate peel from places where medicinal herbs are sold and washed with water to remove suspended impurities and dried at room temperature and milled by an electric mill to obtain pomegranate peel powder, then put 100g of pomegranate peel powder in a thick filter paper thimble and put the solvent in the beaker at the bottom of the chamber consisting of ethanol And water, at a ratio of 40:60, in a quantity of 200ml, at a temperature of 70 o C, for a period of three hours, and then transfer the extract to the evaporator rotary. It was set at a temperature of 60 o C for the purpose of separating ethanol and water from the extract to dry it. The crude extract was placed in an opaque and sealed container and kept in the refrigerator until the compound under study was extracted [9].

The separation of the A1 compound from the extract using a chromatographic column filled with silica gel of the type Mesh size 60 A, which activates and saturates 30 g of gel, using 200ml of a mixture of hexane-methanol (V / V) 1: 5 as it was chosen by giving it the best separation in the Thin Layer Chromatography (TLC), which represents the mobile phase, where 10ml of the extract form was added to the glass separator column, then after the extract was absorbed by silica gel, the column was washed with a 5: hexane-methanol solvent by adding it step by step. Separation within sealed tubes, the solvent was evaporated and dried in an incubator at 37 o C and a portion of the sample was taken for chemical composition detection using Fourier-transform infrared spectroscopy (FTIR) techniques [10].

Extraction and separation of plant extract A2 from green tea: The process of preparing green tea leaf extract included mixing 50g of dry green tea leaves with 500ml of distilled water, after which it was placed in a water bath for 4 hours at a temperature of 50 o C, after which the mixture was filtered using filter paper.

The extract was purified from non-polar compounds by adding chloroform to the extract in a separation funnel in a ratio of 1: 1 (V / V). Extract: chloroform, mix the mixture and leave for an hour, the lower layer (the chloroform layer) was discarded and the upper aqueous layer was purified from the low polar compounds by adding Ethyl acetate to a separating funnel at a ratio of 1: 1 (extract volume: the volume of ethyl acetate), mix the mixture well and leave for 24 hours to obtain two phases where the lower phase represents the aqueous layer (model layer) Concentrate with a rotary evaporator at a temperature of 50 ° C and put the crude extract in a vial. Airtight and refrigerated [11].

Separation of compound A2 from green tea leaf extract by using a column filled with silica gel of the type Mesh size 60 A, which activates and saturates 30g of gel using a mixture of acetic acid - chloroform in a ratio of 1: 9 (V / V) as it was chosen by giving it The best separation is in TLC, which represents the mobile phase, where 10 ml of the extract form was added to the glass separation column, then after the extract was absorbed by silica gel, the column was washed with a solvent -: by adding it step by step and the separated parts were collected at a rate of approximately 5ml of the part contained in the outgoing mobile phase From the separation column inside sealed tubes A2, the solvent was evaporated and a portion of the sample was taken for chemical composition detection using FTIR techniques [10].

Animals used in the study: To know the effect of the compounds extracted from the plants under study, 24 male domestic rabbits obtained from the Animal Center in the College of Veterinary Medicine at Tikrit University were used in this study. The rabbits were placed in cages, taking into account hygiene (cleanliness) and sterilization with disinfectants. Animals used between 1800-2000 g and animals fed with ready-made feed.

Preparation of the feed: The standard feed is used for feeding animals (wheat 35%, yellow corn 35%, soybeans 10%, proteins and other materials) after it was ground using an electric mill, the components of the feed were determined, mixed and kneaded by adding water, then discs were formed from it and placed in large plates to dry with exposure to air and sunlight, then stored in plastic containers [12].

A feed was prepared with similar ingredients to the composition of the standard feed, except that cholesterol was added at a concentration of 1% in

order to induce heart disease in the experimental rabbits [13].

The animals were divided into three groups, each group consisted of eight animals with similar weights, as follows:

The First Group G1: the control group. This group was treated by giving a standard diet and regular drinking water daily during the study period.

The Second Group G2: a group of induced cardiovascular disease, cholesterol, which was dosed with compound A1

This group was treated by administering a standard diet with cholesterol powder at a concentration of 1% and regular drinking water, and after 45 days of disease inception, they were given compound A1 at a concentration of 50 mg / kg by mouth [14].

The Third Group G3: a group of induced cardiovascular disease, cholesterol, which was dosed with compound A2 this group was treated by administering a standard diet with cholesterol powder at a concentration of 1% and regular drinking water, and after 45 days of disease inception in it, compound A2 was administered at a concentration of 6mg / kg orally [15]. After the end of the induction period of cardiovascular disease, blood samples were taken directly through the heart by cardiac stab method from the control group and the two disease induction groups, with an amount of 3ml, then placed directly in the test tubes and inserted into the central centrifuge at 3000rpm for 15 minutes to obtain blood serum, and the effectiveness of the Myeloperoxidase enzyme was measured. And biochemical variables, and blood samples were drawn from the two affected groups, and the compounds under study G2 + A1, G3 + A2 were dosed, serum isolated, and the activity of , myeloperoxidase enzyme and the levels of troponin, potassium and magnesium were measured.

Estimating the effectiveness of the myeloperoxidase enzyme: The effectiveness of the myeloperoxidase enzyme in the blood serum was estimated using a ready-made test kit from the American company Elabscince, where the myeloperoxidase enzyme works on oxidizing ortho-dianisidine in the presence of hydrogen peroxide to produce a yellow-colored compound that can be measured at a wavelength of 460 nm [16,17]

Determination of troponin level: The method of immunological detection is used where the detection antibody in the buffer binds to the antimatter in the sample in the form of antibody complexes with the antigen and is transferred to the nitrocellulose matrix to be picked up by the inhibitor antibody at the end of the test cartridge and the signal intensity on the detection antibody that is processed by Finecare test instrument to demonstrate the concentration of cTn-I in the sample [18]. Potassium ion level estimation: The potassium ion concentration (K^{+1}) in the blood serum was estimated using a ready-made assay kit from the Egyptian company Spectrum, where the potassium ions react with TPB-Na Sodium Ttraphenyl Boron in a basic medium to form TPB- K Potassium Tetraphenyl Boron which is cloudy [19]

Determination of the magnesium ion level: The concentration of the magnesium ion (Mg^{+2}) in the serum was estimated using a ready-made assay kit from the Egyptian company Spectrum, where the magnesium ions form a complex colored compound upon interaction with phosphnazo III and the color intensity is proportional to the magnesium ion concentration [20,21].

Statistical analysis: The result were analyzed statistically by applying the statistical program Minitab (VER/17), the t-test and F (ANOVA) were used and the arithmetic means were compared to determine the differences using Duncan polynomial test with a probability level $P \geq 0.01$.

Results and discussion

Fourier-transform infrared spectroscopy was used to diagnose compounds and to know their structural composition, through hydroxyl groups O-H and carbonyl C = O and other groups [22,23].

The Infrared spectrum of compound A1 as shown in Figure (1) showed a high-intensity stretch beam at 3260-3454 cm^{-1} belonging to the hydroxyl group OH, and a weak-intensity beam at site 3012 cm^{-1} due to the absorption of the aromatic C-H group and an intense beam high at 1706.88 cm^{-1} for the C = O group within the carboxyl group and a bundle with an absorption 1542-1620 cm^{-1} belonging to the benzene aromatic ring[24].

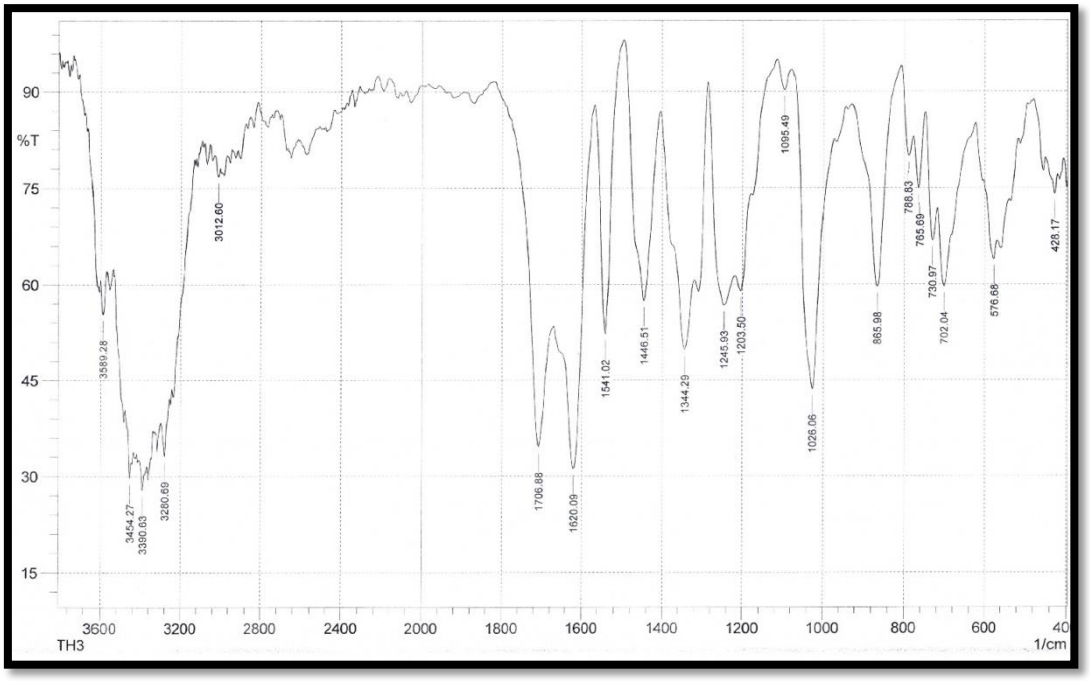

Fig. 1: The infrared spectrum of compound A1

While the FT-IR spectrum of compound A2 as shown in Figure (2) shows a high-intensity beam at 3350.12-3481.27 cm^{-1} for the hydroxyl OH groups associated with the aromatic ring, and a medium-intensity beam at the site of 1645.17 cm^{-1} for the C = O-linked carbonyl group.

With tri-hydroxybenzoic acid and a chroman compound (dihydropyran), as well as a stretch package at 1244.00 cm^{-1} for the O-C = O group and the stretch beam at 1564.16 cm^{-1} for the C = C group in the benzene ring [25,26], and 784.33-883.34cm^{-1} for the C-H aromatic group[27].

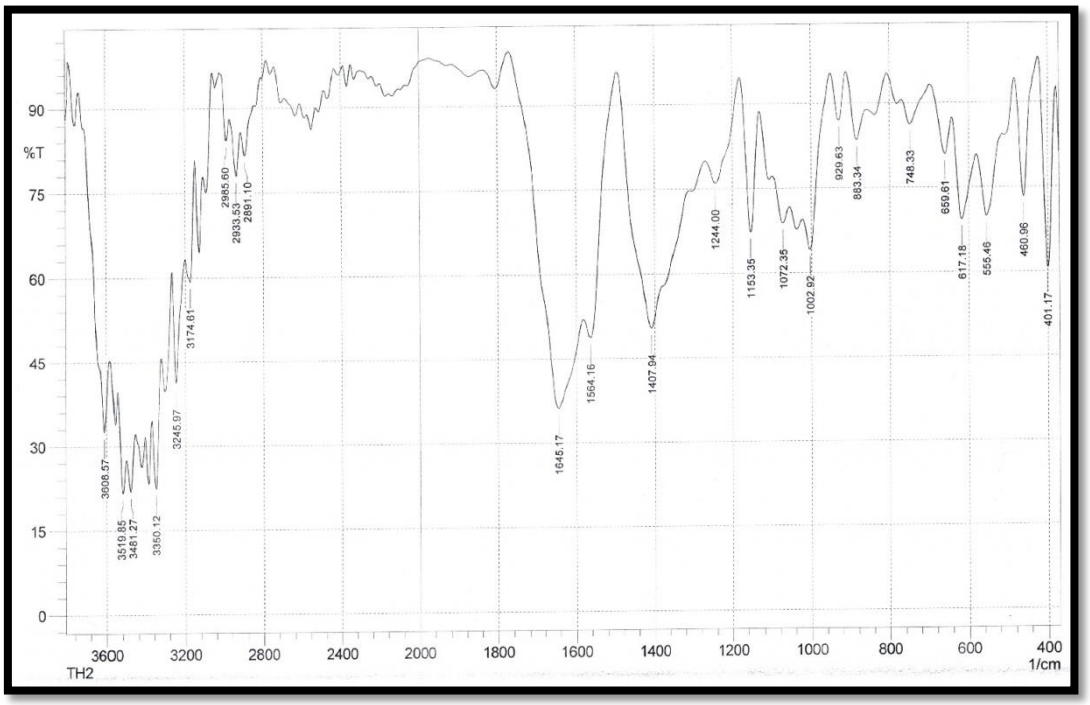

Fig. 2: Infrared spectrum of A2

Estimation of biochemical variables: It is noticed from Table that there is a significant difference in the effectiveness of MPO by the effect of the compounds extracted A1 and A2, as the activity reached (74.138 ±1.095) (60.838 ±0.424) compared to the control group (27.587 ±1.726) and the group in which the disease was developed
(123.500 ±1.938) (123.588 ±2.057), respectively, with the level of probability P≤0.01, and this is consistent with what has been indicated by a number

of studies, where it is observed that there is a decrease in the effectiveness of MPO due to the effect of poly phenol compounds known as flavonoids and phenolic acids (phenolic acid) as it is considered an anti-oxidant and anti-inflammatory and has a preventive effect from heart disease, brain and diabetes, as it works to reduce blood pressure and cholesterol and prevent the accumulation of plaque in the blood vessels by reducing the damage of free radicals in the blood, in addition, the effect of compounds may appear when they are linked with minerals such as iron, thus reducing the production of the hydroxyl radical within the interaction of fenton catalyzed by the MPO enzyme or its association with the active sites, thus reducing its effectiveness or inhibition [28-31].

The results also indicate a significant decrease in the level of troponin due to the effect of the compounds extracted A1 and A2 in the groups G2 + A1 and G3 + A2, where it reached (0.392 ±0.022) (0.1775 ±0.0158) compared to the control group G1 (0.0125 ±0.004) and the group that was developed the disease has G2 and G3 (5.029 ±1.039) (4.760 ±0.572), respectively, with a probability level of P≤0.01 as shown in the Table and this came in accordance with one of the studies that indicated the positive effect of phenolic acids and flavonoids on reducing the level of troponin in the serum of male mice after the development of myocardial infarction in it, by reducing oxidative damage [32].

There was a significant decrease in the potassium level due to the effect of the compounds extracted A1 and A2 in the groups G2 + A1 and G3 + A2, reaching (3.725 ± 0.070) (3.525 ±0.046), compared to the (3.537 ± 0.051) G1 group and the groups in which the disease was developed G2 and G3(4.312 ±0.155) (4.262 ±0.155) respectively, and with a P0.0 probability level, the decrease in the level of potassium in rabbit serum for the G2 + A1 and G3 + A2 groups may be attributed to the effect of the compounds under study on the activity of the HERG channel, as one study indicated that Flavonoids possess an antiarrhythmic effect of QT acquired through potassium channel blocking [33].

The results in the Table indicate that there is a significant difference in the level of magnesium due to the effect of the compounds extracted A1 and A2 in the groups G2 + A1 and G3 + A2, where the efficacy reached (2.650 ±0.016) (2.520 ±0.114) compared to the G1 group (2.205 ± 0.005) There was no significant difference compared with the groups in which the disease was developed, G2 and G3 (2.698 ± 0.025) (2.655 ± 0.114), respectively, and with the probability level P≤0.01. The raise in of magnesium level because of lack of perfusion for along time or because of disturbance in heart rhythm [34] .

Table: The mean ± standard deviation of myeloperoxidase enzyme activity and some biochemical variables rabbit serum for the control group compared with the groups after the induction of myocardial infarction and its treatment with extracted compounds

parameters	Group	N	Mean ± SD	P – value
Myeloperoxidase U/L	G1	8	27.587 ± 1.726 $_c$	0.0002
	G2		123.50± 1.938 $_a$	
	G3		123.58 ± 2.057 $_a$	
	G2+A1		74.138 ± 1.095 $_b$	
	G3+A2		60.838 ± 0.424 $_c$	
Troponin ng / ml	G1	8	0.012 ± 0.004 $_c$	0.0001
	G2		5.029 ± 1.039 $_a$	
	G3		4.760 ± 0.527 $_a$	
	G2+A1		0.392 ± 0.022 $_b$	
	G3+A2		0.177 ± 0.015 $_c$	
Potassium mmol / L	G1	8	3.537 ± 0.051 $_c$	0.0004
	G2		4.312 ± 0.155 $_a$	
	G3		4.262 ± 0.150 $_a$	
	G2+A1		3.725 ± 0.070 $_c$	
	G3+A2		3.525 ± 0.046 $_c$	
Magnesium mg / dl	G1	8	2.205 ± 0.005 $_c$	0.009
	G2		2.698 ± 0.025 $_a$	
	G3		2.655 ± 0.118 $_a$	
	G2+A1		2.650 ± 0.016 $_a$	
	G3+A2		2.520 ± 0.114 $_b$	

Conclusion

1- This present study has showed a significant increase in myeloperoxidase activity and the level of Troponin, Potassium, and Magnesium in serum from rabbits induced with Myocardial Infarction comparing with control group.

2- There was a significant decrease in myeloperoxidase activity, Troponin level and the level of Potassium in serum from rabbits that has dosed the extracted A1,A2 after the disease developed in them.

3- There wasn't significant difference in the Magnesium concentration in serum of rabbits, which was administered by the compounds extracted A1,A2 after the disease developed in these rabbits.

References

[1] Guyton, A.C., and Hall, J.E., (2016),” text book of medical physiology" 13th ed., Philadelphia, Pennsylvania. Esevier Inc. PP:1.

[2] Partha, D., Gabriel, Ying, W., Florian., L., Rostic, G., Clinton , S., et.al. , (2012), Myocardial infraction accelerates atherosclerosis, Nature. 487 (7407): 325-329.

[3] Roger, S., Joanne, M., and Nathan, D., 2011, " Preventive Cardiology: A Companion to Braunwald,s Heart Disease" , 1st ed. Elsevier philadelphia, 1, chap. (2), p:14.

[4] Fredrika, A., Van, N., Michael, S., Cecil, P., Janus, M., Augustinus, D., et al., (2015), Cardiovascular disease risk. JAMA inter. Medi., 175(6): 1007 – 1017.

[5] Patric , T., Bradshaw , J., Nikhil ,K., Susan , L., Teitelbaum, A., Neugut, d., et al., (2016), Cardiovascular disease mortality among breast Cancer survivors , epidemiology Cambridge , Mass ., 27(1): 6-13.

[6] Lavie, C., McAuley P., Church T., Milani R., Blair, S., (2014), Obesity and cardiovascular diseases: implication regarding fitness, and severity in the obesity paradox. J. Am. Coll. Cardiol., 63 (14): 1345 – 54.

[7] Tortora, G., and Bryan Derrickson, (2011),” principles of Anatomy and physiology”, 13th ed.; Hoboken: Jone Wiley & son.

[8] Barnes, J., (2001), An introduction to herbal medicinal products, Pharmacol. J., 268: 804-806.

[9] Al-Mosawe, E., Al-Saadi, (2012), The Extraction and purification of Gallic acid from the pomegranate rind, Al-Mustansiriyah Jour. Sci. 23(6):53-60.

[10] Harborn, J., (1998)” Phytochemical Method” 3rd ed. Chapman &Hall.

[11]-Al-Jumaily, E., Al-abdli, Y., Nasser, N., (2011), Extraction of catechin from green tea plants camellia sinensis, Journal of Biotechnology Research Center, 5(3):9-14.

[12] National Research Council. (1994)” Nutrient requirements of poultry” 9th revised, National academy press, Washington. D. C.

[13] Mohamed, A., Abdelhalim, N., et al. (2008), effects of feeding periods of high cholesterol and saturated fat diet on blood biochemistry and hydroxyproline fraction in rabbits, Bio. Biol. Insights. 2:95-100.

[14]-Choudhury, R., Srai, S., et al. (1999), Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration, Free Radical Biology and Medicine, 27(3-4):278-289.

[15] Bogdanski, P., et al., (2012),Green tea extract reduced blood pressure, inflammatory biomarker, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients, Nut. Res. 32(6):421-427.

[16] Lundquist, I., Josefsson, J., (1971), Sensitive method for determination of peroxidase activity in tissue by means of coupled oxidation reaction, Analytical Biochemistry. 41(2):567-577.

[17] Xia, Y., Zweier, J., (1997), Measurement of myeloperoxidase in leukocyte-containing tissues, Analytical Biochemistry., 245(1):93 -96.

[18] Wong, S., (1996), Strategic utilization of cardiac markers for diagnosis of acute myocardial infarction, Annals of Clinical and Laboratory Science Journal, 26(4):301 -312.

[19] Hilliman, G., et al. (1967), Rapid determination of serum potassium by turbidity measurement with kalignost after protein Precipation, Z kiln. Chem. Klin. Biochem. 5(2):93-94.

[20] Thomas, L., (1998), “Clinical laboratory diagnostic” 1st ed. Frankfurt. Th-Books Verlagsgesellshaft, pp: 231-241.

[21] Mann, C., You, J., (1957), Spectrophotometric determination of Mg Anal. Chem. Acta.16:155-60.

[22] Yuan, C., Jin, Z., et al. (2012), Inclusion complex of astaxanthin with hydroxypropyl-B-cyclodextrin: UV, FTIR, 1HNMR and molecular modeling studies, 89:492-496.

[23] Zhang, J., Li, C., et al. (2011) controllable exploding microcapsules as drug carriers, Chem. Cmmun. 47:4457-4459.

[24] Abri, A., & Maleki, M., (2016), Isolation and identification of gallic acid from the elaeagnus angustifolia leave and determination of total phenolic, flavonoids contents and investigation of antioxidant activity, Inter. Chem. Comm. 4:146-154.

[25] Ferenc, B., Ziegler, M., et al. (2007), Vibrational spectroscopic study on the quantum chemical model and the X- ray structure of gallic acid, solvent effect on the structure and spectra, Vibrational Spectroscopy, 43(1):193-202.

[26] Xu, H., Liu, T., et al. (2019), Interactions between B- cyclodextrane and catechins, and potential anti-osteoclastogenesis activity of the (-)-epigallocatechin-3-gallate –B-cyclodextrane complex, RSC. Advance, 9(48):28006-28018.

[27] Ponnuraj, R., et al. (2015), Formulation and characterization of epigallochatechin gallate nanoparticles, Indo Amer. Jour. Pharm. Rec. 5:387-399.

[28] Tian, R., Ding, Y., et al. (2017), Inhibition of myeloperoxidase and neutrophil-mediated hypochlorous acid formation in vitro and endothelial cell injury by (-)-epigallocatechin gallate, Jour. Agric. Food Chem. 65(15):3198-3203.

[29] Yeoh, B., Olvera, R., et al. (2016), Epigallocatechin-3-gallate inhibition of myeloperoxidase and its counter-regulation by dietary iron and lipocalin 2 in murine model of gut inflammation, Am. J. Pathol. 186(4):912-926.

[30] Kroes, B., et al. (1992), Anti-inflammatory activity of gallic acid, Plant. Med. 58(06):499-504.

[31] Perron, N., et al. (2009), A review of the antioxidant mechanisms of polyphenol compounds

related to iron binding, Cell, Bio. Biophys. 53(2):75-100.
[32] Priscilla, D., et al. (2009), Cardioprotective effect of gallic acid troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in wistar rats. Chem. Biol. Interact. 179(2-3):118-124.
[33] Kelemen, K., et al. (2007), green tea flavonoid epigallocatechin-3-gallate (EGCG) inhibits cardiac hERG potassium channels, Bio. & Biophs. Rese. Comm., 364(3):429-435.
[34] Sora, J., et al (2010), European resuscitation guidelines for resuscitation 2010 section 8. Cardiac arrest in special circumstance: electrolyte abnormalities, poisoning, drowning, accidental hypothermia, hyperthermia, asthma, anaphylaxis, cardiac surgery, trauma, pregnancy, electrocution, Resuscitation, 81(10):1400-1433.

دراسة تأثير المركبات المستخلصة من قشور الرمان والشاي الأخضر على بعض المتغيرات الكيموحيوية في امصال الارانب المستحدث بها احتشاء العضلة القلبية

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الملخص

تهدف هذه الدراسة الى تقصي تأثير المركبات المستخلصة من قشور الرمان والشاي الأخضر على بعض المتغيرات الكيموحيوية في امصال ذكور الارانب المحلية المستحدث بها احتشاء العضلة القلبية.

تمت الدراسة في البيت الحيواني التابع لكلية الطب البيطري، استخدم 24 ذكراً من الارانب وبمعدل أوزان تراوحت بين1800 – 2000 غرام، تم تقسيمها الى ثلاث مجاميع (8 / مجموعة) غذيت المجموعة الأولى عليقة قياسية باعتبارها مجموعة سيطرة وغذيت المجموعة الثانية و الثالثة عليقة قياسية مضاف لها الكوليسترول بنسبة 1% ، وبعد مرور 45 يوماً تم تجريع حيوانات المجموعة الثانية والثالثة التي استحدث فيها احتشاء العضلة القلبية المركبات المستخلصة وقيست فعالية أنزيم مايلوبيروكسيديز ومستوى كل من التروبونين ، البوتاسيوم ، المغنيسيوم.

تم جمع عينات الدم من مجاميع السيطرة والثانية والثالثة التي تم استحداث المرض فيها وبعد 21 يوم جمعت العينات من المجموعتين الثانية والثالثة بعد معاملتها بالمركبات المستخلصة، ان استحداث احتشاء العضلة القلبية أدى الى ارتفاع معنوي في فعالية أنزيم مايلوبيروكسيديز ومستوى التروبونين والبوتاسيوم والمغنسيوم وبمستوى احتمالية $p\leq 0.01$ مقارنةً مع السيطرة، وحصول انخفاض معنوي في فعالية مايلوبيروكسيديز ومستوى التروبونين البوتاسيوم للمجاميع التي جرعت المركبات المستخلصة بعد استحداث المرض فيها وبمستوى احتمالية $p\leq 0.01$ مقارنة مع المجاميع المصابة، وعدم وجود فرق في مستوى المغنسيوم في مجاميع المجاميع المعاملة بالمستخلصات مقارنة مع المجاميع المصابة وعند مستوى احتمالية $p\leq 0.01$.